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# Muscle glycogen utilization during exercise following ingestion of alcohol

Harry A. Smith<sup>1</sup>, Aaron Hengist<sup>1</sup>, Drusus Johnson Bonson<sup>1</sup>, Jean-Philippe Walhin<sup>1</sup>, Robert Jones<sup>2</sup>, Kostas Tsintzas<sup>2</sup>, Gregg H. Afman<sup>3</sup>, Javier T. Gonzalez<sup>1</sup>, James A. Betts<sup>1</sup>.

<sup>1</sup> Department for Health, University of Bath, BA2 7AY, United Kingdom

<sup>2</sup> School of Life Sciences, Queens Medical Centre, University of Nottingham, NG7 2UH, United Kingdom

<sup>3</sup> Department of Kinesiology, Westmont College, CA

**Corresponding Author:** Prof. James A. Betts

Department for Health, University of Bath, Bath, BA2 7AY United Kingdom

Tel: +44 (0) 1225 38 3448

Email: j.betts@bath.ac.uk

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## Abstract

**PURPOSE:** Ingested ethanol (EtOH) is metabolized gastrically and hepatically, which may influence resting and exercise metabolism. Previous exercise studies have provided EtOH via intravenous infusion rather than oral ingestion, which alters the metabolic effects of EtOH. No studies to date have investigated the effects of EtOH *ingestion* on systemic and peripheral (e.g. skeletal muscle) exercise metabolism.

**METHODS:** Eight men (Mean  $\pm$  SD, Age:  $24 \pm 5$  y; Body Mass:  $76.7 \pm 5.6$  kg; Height:  $1.80 \pm 0.04$  m;  $\dot{V}O_{2peak}$ :  $4.1 \pm 0.2$  L.min<sup>-1</sup>) performed two bouts of fasted cycling exercise at 55%  $\dot{V}O_{2peak}$  for 2-h, with (EtOH) and without (Control) prior ingestion of EtOH 1-h and immediately before exercise (total dose: 0.1 g·kg lean body mass<sup>-1</sup>·h<sup>-1</sup>;  $30.2 \pm 1.1$  g 40% ABV Vodka; fed in 2 equal boluses) in a randomized order, separated by 7-10 days.

**RESULTS:** Muscle glycogen breakdown during exercise was not different between conditions (Control: -257.7 [-330.8, 184.6] vs EtOH: -221.4 [-287.6, 141.4] mmol·kg dm<sup>-1</sup>; means with normalized 95% confident intervals). Mean plasma glucose concentrations during exercise were similar (Control: 5.26 [5.17, 5.34] vs EtOH: 5.26 [5.18, 5.34] mmol·L<sup>-1</sup>; *p* = 0.04). EtOH ingestion resulted in similar plasma non-esterified fatty acid (NEFA) concentrations compared to rest (Control: 0.43 [0.31,0.55] vs EtOH: 0.30 [0.21,0.40] mmol·L<sup>-1</sup>) and during exercise. Mean plasma lactate concentration was higher during the first 30-min of rest following EtOH consumption (mean concentration: Control: 0.83 [0.77, 0.90] vs EtOH 1.00 [0.93, 1.07] mmol·L<sup>-1</sup>) but the response during exercise was similar between conditions.

**CONCLUSIONS:** Ingesting a small dose of EtOH transiently altered resting concentrations of systemic lactate, but not during exercise. Muscle glycogen utilization was similar during exercise with or without prior alcohol ingestion, reflected in similar total whole-body carbohydrate oxidation rates observed.

## 55    **Introduction**

56    Ethanol (EtOH) is the relatively energy-dense ( $\sim 7.1 \text{ kcal.g}^{-1}$ ) ingestible form of  
57    alcohol and can be preferentially oxidized over other nutrients [1-3]. Low doses  
58    are primarily metabolized through the action of alcohol dehydrogenase (ADH)  
59    and aldehyde dehydrogenase (ALDH) in the gut and the liver. The resultant  
60    reduction in the cellular  $\text{NAD}^+:\text{NADH}$  redox ratio disturbs metabolic pathways in  
61    the liver that either require  $\text{NAD}^+$  or are inhibited by NADH [4]. Specifically, this  
62    includes pathways vital for energy turnover (i.e. glycolysis, citric acid cycle,  
63    pyruvate dehydrogenase, fatty acid oxidation, and gluconeogenesis). At the  
64    whole-body level, maximal rates of EtOH oxidation ( $0.1 \text{ g.kg}^{-1}\text{h}^{-1}$  lean body mass)  
65    have been suggested to transiently spare the oxidation of other substrates (i.e.  
66    carbohydrate and fat) up to a maximum level of half an individual's resting  
67    metabolic rate [5, 6]. However, it remains unclear whether substrate sparing  
68    manifests during times where the requirement for energy turnover is high (i.e.  
69    during exercise).

70        Necessarily, any alterations in circulating concentrations of metabolic  
71    substrates as a result of EtOH ingestion could influence metabolic fuel selection  
72    during exercise [7]. Specifically, as muscle glycogen depletion is a largely  
73    dictated by the total amount of carbohydrate available to the system [8],  
74    preferential oxidation of EtOH over carbohydrate may spare carbohydrate at the  
75    systemic level during moderate intensity exercise; resulting in sparing of muscle  
76    glycogen concentrations during a bout of exercise. However, the influence of  
77    EtOH on circulating glucose and non-esterified fatty acids (NEFA) during exercise  
78    remains indistinct [9-12]. Discrepancy in findings between studies may be

explained by inconsistencies in dose of EtOH, with a greater effect being observed following larger relative doses (~20-40 g) [9, 12]. Similarly, EtOH blunts the typical blood glucose response to exercise during moderate (>50%  $\dot{V}O_{2max}$ ) [9, 13], but not lower intensity exercise (30%  $\dot{V}O_{2max}$ ) [10].

Importantly, the majority of exercise studies to date have investigated the influence of EtOH on carbohydrate and fat metabolism following infusion, rather than ingestion, which could alter the extent of displacement of hepatic, and therefore skeletal muscle carbohydrate and fat metabolism during exercise [14-18]. Furthermore, whilst it has been generally accepted that exercise *per se* will not increase the rate of EtOH metabolism [19], the 2-fold elevation in hepatic  $\dot{V}O_2$  during exercise (~60 mL.min<sup>-1</sup> at rest vs ~135 mL.min<sup>-1</sup> during exercise), suggests the increase in liver metabolic rate could augment EtOH metabolism especially when fed a dose within the liver's capacity to oxidize EtOH [20-22]. No studies to date have investigated the effects of EtOH *ingestion* on systemic and peripheral (e.g. skeletal muscle) metabolism during exercise.

Based on previous work it was expected that a low dose of orally ingested EtOH would alter circulating metabolites thereby meaningfully displacing carbohydrate and fat oxidation at rest and during exercise, sparing skeletal muscle glycogen utilization. Therefore, the objective of the current study was to investigate the whole body metabolic and skeletal muscle glycogen responses to acute ingestion of a dose of EtOH estimated to maximally stimulate hepatic oxidation, prior to a bout of prolonged, moderate-intensity exercise in young, healthy men.

## **Materials and Methods**

### ***Experimental design***

Participants performed two bouts of cycle ergometry in a randomized cross-over design, interspersed by an interval of 7-10 days. A dietary record was collected during the 48-hour period prior to the first experimental trial and was subsequently replicated with exact types and amounts of foods before the final trial ( $2726 \pm 490$  kcal·day<sup>-1</sup>,  $47 \pm 3$  % carbohydrate,  $33 \pm 8$  % fat,  $21 \pm 9$  % protein). Furthermore, participants were asked not to perform vigorous physical activity, consume alcohol, or caffeine 24-h prior to testing, confirmed by questionnaire upon entering the laboratory. Main trials involved ingestion of EtOH beverages or volume-matched water as a control, followed by 1-hour of rest and then 2-hours cycling at 55% of individual  $\dot{V}O_{2peak}$ . The study randomisation plan was created using <https://www.random.org/>.

### ***Participants***

Seven healthy recreationally active men (**Table 1**) were recruited to participate in the study. Participants were informed of potential risks and discomfort involved in the study prior to providing written informed consent. The study was approved by the National Health Service Research Ethics Committee: Bristol (17/SW/0219), the Research Ethics Approval Committee for Health at the University of Bath (EP 17/18 090) and was registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT03404947). All procedures were performed in accordance with the Declaration of Helsinki.

### ***Preliminary measurements***

Prior to experimental sessions participants visited the human performance laboratory at the University of Bath for fitness and body composition analysis. Body mass was assessed to the nearest 0.1 kg using electronic weighing scales (BC543 Monitor; Tanita, Netherlands) and height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany), before lean and fat mass were determined using dual-energy X-ray absorptiometry (DEXA; Discovery, Hologic, Bedford, UK). An incremental cycling test was then completed on an electronically braked ergometer, at a self-selected cadence, to assess maximum oxygen uptake ( $\dot{V}O_{2peak}$ ) (Excalibur Sport; Lode®, Netherlands). Participants were permitted to adjust the handlebar and saddle heights to their preference. Power output was initially set at 50 Watts (W), increasing in 50 W increments every 4 minutes for four stages. Thereafter, power output increased in 20-W increments every 1-minute until volitional exhaustion was achieved. Heart rate was monitored throughout (Polar H7; Polar Electro, Finland) and breath-by-breath assessment of  $\dot{V}O_2$  was made using an online gas analysis system (TrueOne2400; Parvomedics, USA). Volume and gas analysers were calibrated with a 3-litre calibration syringe (Hans Rudolph, USA) and known concentrations of a calibration gas (15.99% O<sub>2</sub>; 5.08% CO<sub>2</sub>). Peak oxygen uptake ( $\dot{V}O_{2peak}$ ) was recorded as the highest average  $\dot{V}O_2$  (L.min<sup>-1</sup>) over a consecutive 30-s period. Peak power output ( $W_{peak}$ ) was calculated using the following equation [23].

$$W_{peak} = W_{final} + \left(\frac{t}{T} \cdot W_{increment}\right)$$

Where  $W_{peak}$  is peak power output (Watts),  $W_{final}$  is the power output of the final completed stage (Watts),  $t$  is time completed of the final stage (s),  $T$  is total stage

time (s), and  $W_{increment}$  is the power increment between stages at exhaustion (Watts).

### ***Experimental protocol***

Participants arrived at the laboratory in an overnight fasted state (~10 h). Body mass was assessed (Sliding Beam Column Scale, Weylux, UK) before participants were instructed to rest in a semi-supine position (~60°), prior to the assessment of resting metabolic rate (RMR) via the Douglas bag technique. Collection of expired gas through the Douglas bag technique allows for calculation of oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ), thereby allowing for calculation of whole-body carbohydrate and fat oxidation [24]. An intravenous cannula was placed into an antecubital vein and a baseline sample of 5 mL venous blood was collected (BD Venflon Pro; BD, Switzerland). Cannulae were kept patent by flushing with 0.9% sodium chloride infusion (B. Braun; UK). Participants then ingested 50% of the total EtOH (Absolut Raspberri, 40% ABV; Absolut, Sweden) or volume matched water before a 60-minute rest period during which venous blood samples were collected at 15, 30, 45 and 60 minutes, alongside a 10-min gas sample between 50-60 minutes. Participants then remained in a semi-supine position while muscle was sampled from the *vastus lateralis* under local anaesthetic (1% lidocaine; Hameln Pharmaceuticals Ltd., Brockworth, UK). Samples were taken immediately prior to the second bolus of EtOH, from a 3-5 mm incision in the anterior aspect of the thigh using a Bergstrom needle adapted for suction and were snap-frozen in liquid nitrogen for subsequent storage at -80°C [25]. A second incision was also made and temporarily dressed for immediate post-exercise muscle sampling. Thereafter,



participants were asked to ingest the remaining 50% of the EtOH or control beverage, immediately prior to the initiation of exercise. Participants were then asked to cycle for 2-hours at 55% of their pre-determined  $\dot{V}O_{2peak}$  on a cycle ergometer (Monark 894E; Monark, Vansbro, Sweden). One-minute expired breath, and 5-ml venous blood samples were collected, alongside heart rate (Polar H1; Polar Electro, Kempele, Finland), every 15-minutes for the first hour and every 30-minutes during the second hour (Figure 1). In both trials, throughout exercise participants ingested water at a rate of 0.5 mL.kg<sup>-1</sup> every 10-minutes (Total: 376 ± 26 ml). Immediately post exercise, participants were transferred from the ergometer to the bed, where the post-exercise muscle sample was collected.

### ***Test beverage composition***

The rate of EtOH ingestion in the EtOH trial was 0.1 g.kg LBM<sup>-1</sup>.h<sup>-1</sup> [6], which aimed to provide sufficient EtOH to contribute meaningfully as a metabolic substrate but without intending to exceed the maximum rate of EtOH metabolism and unnecessarily overspill into systemic circulation [6]. As such, total EtOH provided was 12.1 ± 0.4 g ingested as a 15% solution in water (30.2 ± 1.1 g 40% ABV Vodka; ~67 kcal). In the control trial, participants ingested a volume matched water beverage.

### ***Blood analysis***

Blood samples were immediately transferred into tubes treated with ethylenediaminetetraacetic acid (EDTA) prior to being centrifuged at 3466 g (5000 rpm) for 10 minutes at 4°C (Heraeus Primo R; Thermo Fisher Scientific, UK) and frozen on dry ice for storage. All samples were later analyzed for plasma glucose (colormetric), non-esterified fatty acids (colormetric), lactate

(colormetric), and a subset of samples for EtOH (colormetric) using a spectrophotometric analyser (RX, Daytona, Randox Laboratories Ltd., UK). Inter-assay CV was < 3% for glucose, < 7% for NEFA, and < 3% for lactate. Intra-assay CV was < 2% for glucose, < 5% for NEFA, and < 3% for lactate.

### **Muscle analysis**

Frozen muscle samples were placed in a freeze dryer (Mechatech Systems, UK) for ~16 hours at -55°C. Following removal of visible connective tissue, freeze-dried muscle samples were reduced to a fine powder using a pestle and mortar and then used to determine muscle glycogen concentrations. Briefly, the muscle powder was digested in 0.1 mM NaOH and neutralized with HCl-citrate buffer, pH = 5.0. The glycogen present in the supernatant was hydrolyzed with  $\alpha$ -amylglucosidase and analyzed for glucosyl units in duplicate by an enzymatic method [26]. Relative concentrations of muscle glycogen were assessed in duplicate using a spectrophotometric plate reader (SpectraMax 190, Molecular Devices, USA). To account for possible measurement error associated with fluid shift during exercise, glycogen concentrations are reported as mmol glucosyl units per kilogram of dry mass ( $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dm}^{-1}$ ). Total rates of muscle glycogen utilization in relation to whole-body carbohydrate oxidation were then calculated as the change in glycogen content of the *vastus lateralis* ( $\text{g}\cdot\text{kg}^{-1}$  dry mass $\cdot\text{min}^{-1}$ ) multiplied by the estimated active muscle mass during exercise, which is almost exclusively the thigh muscles for seated cycling and can be estimated as ~10 kg wet mass in young men [27].

## **Expired gas analysis**

Inspired air concentrations were assessed during Douglas bag collection to correct for changes in atmospheric O<sub>2</sub> and CO<sub>2</sub> concentrations [28]. Expired gas concentrations of O<sub>2</sub> and CO<sub>2</sub> were analysed in a known volume of sample, using paramagnetic and infrared analysers, respectively (Mini HF 5200; Servomex Group Ltd, Crowborough, UK). Total volumes of expired gas were determined using a dry gas meter (Harvard Apparatus, Holliston, USA) and temperature measured using a digital thermometer (Edale Instruments, Longstanton, UK). Substrate utilization during exercise was determined using the equations of Jeukendrup and Wallis (2005) [29] (where  $\dot{V}O_2$  and  $\dot{V}CO_2$  are expressed in L·min<sup>-1</sup>). EtOH oxidation was assumed to be complete, based on feeding at the maximal hepatic oxidation rate reported in Schutz [6]. Measured RER was adjusted for EtOH oxidation to give Non-EtOH RER. This was calculated by subtracting the  $\dot{V}O_2$  and  $\dot{V}CO_2$  associated with complete oxidation of ingested EtOH (i.e. 3O<sub>2</sub> & 2CO<sub>2</sub> per mol EtOH respectively) from measured values of  $\dot{V}O_2$  and  $\dot{V}CO_2$ .

## **Statistical analysis**

All data in the text are reported as means [normalized 95% confidence interval] unless otherwise stated. Normality of data was assessed using the Shapiro-Wilk test, with a paired t test or Wilcoxon's test employed to analyse parametric data and non-parametric data respectively. A mixed model ANOVA (condition, time, and condition x time) was used to examine differences in plasma metabolite in data, with post-hoc Bonferroni corrections applied in GraphPad Prism (GraphPad Software Inc., California, USA). Effect sizes (Cohen's *d*) were

calculated and interpreted in accordance with [30]. Error bars shown on figures are confidence intervals (CI) corrected for inter-individual variation using the specific error term from the pairwise contrast at each time-point [31]. Rather than describing the variability of individual values around the mean in each condition, the magnitude of these confidence intervals provides a visual representation of the contrast between means such that, in general, plotted means whose confidence intervals overlap by no more than half one side of an interval would typically generate a  $p$ -value less than 0.05 if using a paired t-test at that time-point [32]. There was no evidence of trial order effects for any variable, which was verified using 2-way ANOVA of Sequence x Condition interactions (all  $p = 0.2$ - $0.9$ ). Based on differences in arterial glucose concentrations observed in Juhlin-Dannfelt *et al* [11] a sample size of 10 participants was deemed sufficient to provide an 80% chance of detecting such a difference at an alpha level of 0.05 (G Power 3.1). Statistical analyses were performed using SPSS Statistics v.24 (IBM Corp., Armonk, NY, USA) and figures were created using GraphPad Prism v.7 (GraphPad Software, San Diego, CA, USA).

## Results

### ***Muscle glycogen and substrate metabolism***

Pre-exercise muscle glycogen content was not different between EtOH and Control conditions (471 [387, 555] vs 469 [385, 553] mmol·kg dm<sup>-1</sup> respectively;  $p = 0.86$ ) and muscle glycogen used during exercise was also not clearly different between trials (EtOH: 229 [156, 302] vs CONTROL: 258 [185, 331] mmol·kg dm<sup>-1</sup>;  $p = 0.67$ ) (Figure 2A).

ANOVA revealed main effects for time (both  $p < 0.01$ ) but not condition ( $p = 0.85$  &  $0.35$ ), or time x condition ( $p = 0.30$  &  $0.30$ ) for  $\dot{V}O_2$  and  $\dot{V}CO_2$  respectively (Figure 3A & 3B). No effect was seen for time ( $p = 0.30$ ), condition ( $p = 0.94$ ), or time x condition ( $p = 0.24$ ) for raw respiratory exchange ratio (RER) (Figure 3C). When adjusted for complete EtOH oxidation, resting RER increased following EtOH consumption but was not different from the control condition ( $1.03$  [ $0.92, 1.15$ ] vs  $0.91$  [ $0.79, 1.02$ ]), with no effects of time ( $p = 0.22$ ), condition ( $p = 0.21$ ), or time x condition ( $p = 0.24$ ) (Figure 3D).

Extra-muscular carbohydrate, and therefore total whole-body carbohydrate oxidation was similar following EtOH ingestion when compared to the Control condition ( $P = 0.56$ ) (Figure 2B). Total fat oxidation during exercise was also similar following EtOH ingestion compared to the control trial ( $1.42$  [ $1.12, 1.72$ ] vs  $1.71$  [ $1.41, 2.01$ ] mJ, respectively;  $p = 0.33$ ).

### **Systemic metabolites**

*Plasma Glucose Concentrations* - Pre-ingestion plasma glucose concentrations were similar between EtOH and Control conditions ( $5.38$  [ $5.29, 5.46$ ] mmol·L<sup>-1</sup> and  $5.31$  [ $5.22, 5.39$ ] mmol·L<sup>-1</sup>, respectively). No effect of time ( $p = 0.13$ ), condition ( $p = 0.47$ ), or time x condition ( $p = 0.63$ ) for plasma glucose response was revealed.

*Plasma NEFA Concentrations* - Baseline (resting) plasma NEFA concentrations were similar between conditions (Figure 4). ANOVA revealed main effects for time ( $p < 0.01$ ), but not condition ( $p = 0.40$ ), or time x condition ( $p = 0.34$ ) for plasma NEFA responses.

*Plasma lactate Concentrations* – Main effects of time ( $p < 0.01$ ), but not condition ( $p = 0.15$ ), or time x condition ( $p = 0.40$ ) were revealed for plasma lactate responses (Figure 4). However, across the first 30-mins of the rest period plasma lactate was higher following EtOH ingestion relative to the control condition (mean concentration: Control: 0.83 [0.77, 0.90] vs EtOH 1.00 [0.93, 1.07] mmol·L<sup>-1</sup>;  $p = 0.04$ ,  $d = 0.77$ ).

*Plasma EtOH Concentrations* - Plasma EtOH remained below the lower detectable limit of the assay (0.72 mmol·L<sup>-1</sup>; EtOH, Randox Laboratories Ltd., UK) throughout the entire protocol.

### ***Exercise intensity***

Average power output (Mean  $\pm$  SD) for the cycling exercise was 156  $\pm$  14 W at a self-selected cadence of 80  $\pm$  9 rpm. Average heart rate during exercise was 154 [150, 157] bpm in the EtOH trial and 157 [154, 161] bpm in the control trial.

### **Discussion**

This is the first study to investigate exercise metabolism following oral alcohol ingestion, with measurements made at the level of skeletal muscle, systemic metabolites and whole-body substrate oxidation. Accordingly, we report the novel observations that prior alcohol ingestion does not alter the utilization of either muscle glycogen or extra-muscular carbohydrate sources during exercise. However, at rest the ingestion of alcohol did increase plasma lactate concentrations. During the subsequent exercise, whole-body fat oxidation was then lower following EtOH ingestion. These metabolic effects were apparent with

a relatively low dose of vodka within the capacity for alcohol metabolism and thus without any measurable appearance of EtOH in the systemic circulation.

Interestingly, ingestion of  $0.1 \text{ g EtOH} \cdot \text{kg LBM}^{-1} \cdot \text{h}^{-1}$  ( $12.1 \pm 0.4 \text{ g}$ ) did not result in differing muscle glycogen use relative to water ingestion in the current study. This is consistent with the one previous study to have quantified muscle glycogen use during exercise under conditions where EtOH is present in the circulation [11]. Despite demonstrating a reduction in muscle glycogen at rest following EtOH infusion, the latter study by Juhlin-Dannfelt and colleagues [11] found infusion of EtOH did not result in differing muscle glycogen utilisation during exercise. The relatively low dose of EtOH ingested in the current study (~30 mL of 40% ABV vodka) was intended not to exceed the capacity for gastric/hepatic metabolism such that EtOH would not appear systemically, so it remains a possibility that a higher dose may stimulate hepatic lipogenesis and/or suppress hepatic glucose production sufficient to modify the balance of skeletal muscle fatty acid, glucose and thus glycogen utilization [6]. Notably, one participant's glycogen use was near zero, so caution must be taken in interpreting absolute values which depend on assumed constants, albeit the relative pattern would not be systematically altered between treatments.

Notably, the current study was the first to assess substrate metabolism using indirect calorimetry during both rest and exercise following EtOH ingestion. Previous studies propose that acute ingestion of EtOH at rest transiently spares the oxidation of carbohydrate and fat up to a maximum level of half an individual's resting metabolic rate (~15-43% sparing of carbohydrate and ~30% sparing of fat oxidation) [5]. RER adjusted for EtOH oxidation was similar between conditions

with initial decreases over rest likely reflective of a shift towards the RER for EtOH (~0.66) in the EtOH trial, and greater lipid oxidation in the Control condition [33]. Likewise, when adjusted for assumed complete EtOH oxidation, resting RER was not different between conditions during rest or exercise, reflected in similar whole body extra-muscular carbohydrate, and fat oxidation (Figure 2A).

Following an approximate 10-hour overnight fast, plasma glucose concentrations were similar at rest between conditions in the current study. Previous research has demonstrated that at rest, EtOH results in either a reduction, or no change in blood glucose concentration, with conflicting results primarily explained by the nutritional status of participants. The current study therefore agrees with previous literature demonstrating no influence of EtOH on resting blood glucose concentrations in humans following an overnight fast of ~12-hours [34-36]. However, ingestion of EtOH lead to higher plasma lactate concentrations during the first 30-mins of the rest period relative to the control group, consistent with the possibility that EtOH can inhibit the conversion of lactate to glucose, and further research should seek to measure this [34, 37]. Whilst blood glucose homeostasis may have been maintained through a compensatory rise in hepatic glycogenolysis in this scenario [34], such a rise in lactate is still well within the normal physiological range, such that any compensatory increase in hepatic glycogenolysis is likely to be minimal.

Plasma concentrations of NEFA were also similar between conditions in the current study, which is likely due to the relatively small dose of EtOH ingested by participants [38, 39]. Notably, the area under the curve response of acetate to EtOH is dose-dependent and whilst the current study did not assess the plasma



acetate responses, it is likely that EtOH ingestion at the dose fed in the current study was not sufficient to elevate plasma acetate concentration and therefore inhibit adipose tissue lipolysis [39, 40].

During bouts of exercise performed in the fasted state, elevation in hepatic gluconeogenesis maintains delivery of glucose to the working muscles [7]. Following ingestion of a small dose of EtOH fed in two boli, blood glucose concentration during exercise did not differ between conditions in the current study. Moreover, both plasma lactate and NEFA concentrations did not differ between conditions during the 2-h exercise protocol. Interestingly, the responses of circulating metabolites during exercise were remarkably similar, suggesting that any observed effect of acute EtOH ingestion at rest is not present during exercise. Bearing in mind that hepatic  $\dot{V}O_2$  increases over two-fold during exercise ( $\sim 60 \text{ mL}\cdot\text{min}^{-1} \rightarrow \sim 135 \text{ mL}\cdot\text{min}^{-1}$ ), and that disposition and first pass metabolism of EtOH is affected by liver function, the increase in liver metabolic rate *may* have augmented EtOH metabolism in the current study [20, 21].

## **Conclusion**

A low pre-exercise dose of alcohol within the capacity for complete oxidation does not meaningfully alter oxidation of fat or carbohydrate during subsequent exercise.

## **Acknowledgements**

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## Conflict of Interest

The authors have no conflicts of interest to declare. The results of the present study results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation and do not constitute endorsement by the American College of Sports Medicine (ACSM).

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**Figure 1.** Schematic representation of the experimental protocol. EtOH = Ethanol.  $\dot{V}O_{2max}$  = maximal oxygen uptake. n=7.

**Figure 2.** Mean  $\pm$  normalised 95% confidence interval A. Substrate contribution to energy expenditure (left) and individual muscle glycogen responses (right). EtOH was assumed fully oxidised, lipid oxidation was calculated from non-EtOH RER, Extra-muscular carbohydrate oxidation was calculated as the difference between whole-body carbohydrate oxidation and measured muscle glycogen utilisation. B. Pre and post exercise muscle glycogen content following EtOH ingestion and volume matched, non-caloric Control. n=6.

**Figure 3.** Mean  $\pm$  normalised 95% confidence interval **A.** Raw  $\dot{V}O_2$  **B.** Raw  $\dot{V}CO_2$  **C.** Raw respiratory exchange ratio **D.** Non-EtOH adjusted respiratory exchange ratio at rest and during exercise following EtOH ingestion and volume matched, non-caloric Control. n=7.

**Figure 4.** Mean  $\pm$  normalised 95% confidence interval Plasma Glucose, Lactate, and Non-esterified fatty acid (NEFA) responses to EtOH ingestion at rest and during exercise compared to a volume matched, non-caloric Control. \* $p < 0.05$ . n=7.









